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14. ABSTRACT

The purpose of this project is to test the hypothesis that food allergy may develop in response to antigen inhalation. Studies in a mouse model are determining the importance of: 1) lung inflammation; 2) dose of inhaled antigen; 3) interactions among inhaled antigens; and 4) the relative timing of antigen ingestion vs. antigen inhalation to lead to food allergy development. We are also testing whether exposure to aerosolized antigen will reverse or exacerbate established food allergy to that antigen. Studies in year 1 of this project demonstrate that: 1) initial inhalation of the potent aeroallergen, house dust mite (HDM), along with egg white (EW), increases the severity of allergic airway disease that develops to further inhalation of EW, but does NOT food allergy induced by EW ingestion; 2) intravenous injection of mice with IgE antibody to trinitrophenyl (TNP), followed by oral challenge with TNP-bovine serum albumin (TNP-BSA) induces shock (hypothermia), but not diarrhea; 3) repeated injection of IgE anti-TNP, followed by challenge with TNP-BSA causes loss of the hypothermia response and generation of an IgG anti-TNP antibody. Finally, we have generated large quantities of monoclonal antibodies to IL-10R, TGF-β and CD25, which will be used in our future studies. These studies can suggest improved strategies for preventing food allergy development, and possibly, for reversing established food allergy.

15. SUBJECT TERMS
Food allergy, asthma, IgE, mast cell, peanuts, egg white, antibody, house dust mite, antigen, cytokine

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1. Introduction: Food allergy is a substantial and growing problem, that affects 4% of the adult population in the US. The mechanisms responsible for the development of food allergy are uncertain; in fact, the very development of food allergy is paradoxical, because allergen ingestion generally induces tolerance rather than an immune response. The purpose of this project is to test the hypothesis that food allergy may develop in response to antigen inhalation, rather than antigen ingestion. Studies are being performed in a mouse model to test this hypothesis. More specifically, we are determining the importance of: 1) lung inflammation; 2) dose of inhaled antigen; 3) interactions among different inhaled antigens; and 4) the relative timing of antigen ingestion vs. antigen inhalation to lead to the development of food allergy. In addition, we test whether exposure to aerosolized antigen will reverse or exacerbate established food allergy to that antigen. These studies can suggest improved strategies for preventing food allergy development, and possibly, for reversing established food allergy.

2. Key Words: Food allergy, asthma, IgE, mast cell, peanuts, egg white, antibody, house dust mite, antigen, cytokine

3. Major Goals:

Aim 1. Determine the conditions under which inhalation of aerosolized egg white can prime for development of food allergy to egg white. Timeframe: months 1-20

Task 1. *Determine whether inflammation induced by aspiration of saline would allow exposure to aerosolized egg white to induce allergic airway disease and/or prime for food allergy* (months 1-8).

Task 2. *Determine whether induction of allergic airway disease and/or priming for food allergy requires airway deposition of a higher dose of egg white than is accomplished by our aerosol protocol* (months 1-12).

Task 3. *Determine whether induction of allergic airway disease by inhalation of an unrelated allergen will allow exposure to aerosolized egg white (EW) to prime for food allergy (FA)* (months 13-20).

Aim *2*. *Determine whether airway-mediated induction of food allergy by one antigen increases the ability of a second, unrelated antigen to induce food allergy* (months 1-12).

Aim 3. *Determine whether ingestion of egg white will inhibit the ability of egg white inhalation to prime for development of egg white food allergy months 13-24.*

Aim 4. *Test the hypothesis that food regurgitation and aspiration may prime for food allergy. Timeframe: months 13-24.*

Task 1: Determine the best time after feeding to recover partially digested egg white from the stomach (month 13).

Task 2: Perform a dose-response study that compares the abilities of fresh egg white vs. stomach-recovered egg white to induce allergic airway disease and initiate food allergy when inoculated intratracheally (months 14-24).

Aim 5. *Determine whether airway priming with birch pollen can induce murine food allergy to apple and celery (months 25-36).*

Aim 6. *Determine whether inhalation of aerosolized egg white can reverse established egg white food allergy. Timeframe: months 1-36.*

Task 1: Determine whether inhalation of low doses of aerosolized egg white can suppress established food allergy to this antigen (months 25-35).

Task 2: Histological evaluation of lungs from the same mice used in task 1 to determine effects of the aerosolized egg white on airway inflammation and fibrosis (months 29-35).

Task 3: Produce mAbs to IL-10R, TGF-β and CD25, which will be used in Aim 1, task 2, Aim 3 and Aim 6 task 3 (months 1-36).

Task 4: Determine whether mAbs to TGF-β, the IL-10R and/or CD25 will block the induction of tolerance by aerosolized egg white (MONTHS 25-36).

Accomplishments:

The major goals of months 1-12 were to: 1) determine the conditions under which inhalation of aerosolized egg white leads to tolerance to egg white vs. priming for the development of egg white allergy (Aim 1, tasks 1 and 2); and 2) determine whether induction of food allergy by one allergen enhances the development of food allergy to a second allergy (Aim 2). They also included production of monoclonal antibodies to be used to determine the mechanisms involved in tolerance induction. We have only been partially successful in meeting these goals. This was caused by a problem with our standard protocol for inducing the development of food allergy to egg white. Our preliminary data indicated that intratracheal inoculation with egg white (3 times a week for 3 weeks), followed by oral gavage with egg white (3 times a week for up to 4 weeks), induced food allergy to egg white, as demonstrated by the development of shock (detected as hypothermia) and diarrhea following egg white ingestion. All planned experiments require the use of this model. However, when we initiated the planned experiments, we had difficulty reproducing these observations. Analysis of our data indicated that intratracheal inoculation of mice with egg white continued to induce allergic airway disease (necessary for priming mice to develop food allergy), but did not induce as severe allergic airway disease as we had previously observed (this may have been because of construction in our mouse colony (subsequently completed) that stressed the mice in the colony, which can have immunosuppressive effects). Because of this, we took the approach described in our Aim 2. That is, we evaluated whether the intratracheal inoculation of mice with both egg white and a more potent allergen that is commonly associated with asthma, house dust mite), would increase the severity of allergic airway disease, and by doing so, increase priming for the development of egg white food allergy. The protocol used involved inoculating mice twice intratracheally with a combination of egg white and house dust mite, then inoculating them 3 times a week for 6 weeks with egg white alone. We also performed this experiment with two different investigators inoculating the mice, to determine whether differences in technique might account for different results. The results of this study were clearcut. As shown in Figure 1, below, mice that had initially received both egg white and house dust mite intratracheally developed considerably more severe allergic airway disease (as detected by an increase in enhanced pause (Penh), a measurement of changes in breathing pattern, in response to inhalation of methacholine). Identical results were obtained by the two investigators.

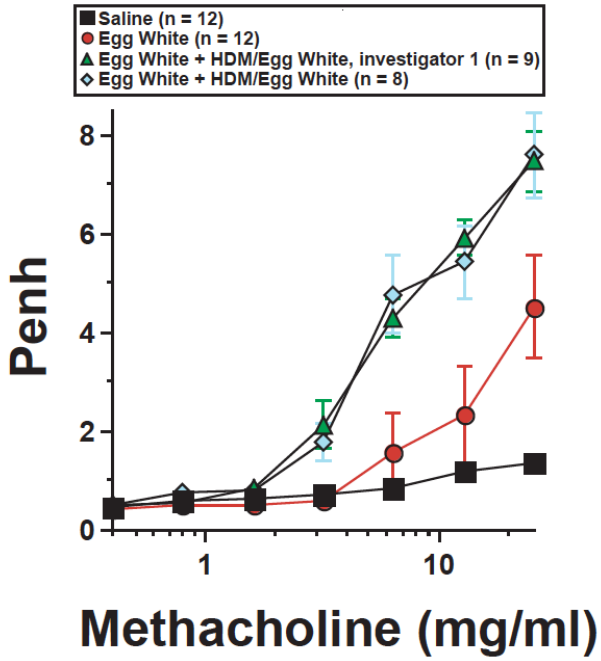
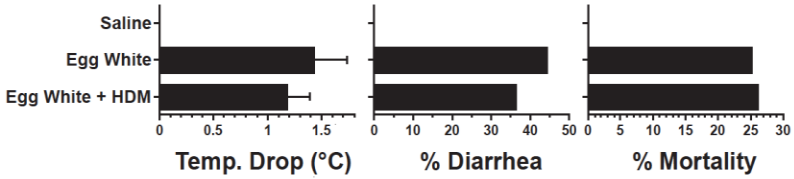


Figure 1. House dust mite (HDM) inoculation enhances the development of allergic airway disease to egg white. BALB/c mice were inoculated twice intratracheally with saline, HDM plus egg white or egg white alone. Following this, all mice that had initially been inoculated with saline were inoculated 17 times intratracheally 3x/week with saline, while all other mice were inoculated intratracheally 3x/week with egg white alone and tested, 1 day after the last egg white inoculation, for responsiveness to methacholine, as measured by enhanced pause (Penh) by non-invasive barometric plethysmography. Means and standard errors are shown. Numbers of mice per group (n) are shown in parantheses.

This result makes the important point (the main objective of our aim 2) that development of an allergic response to a potent allergen acts amplifies the allergic response that develops to a less potent allergen that is initially co-administered with the more potent allergen. Put into a "real world" context, it suggests that individuals who have allergic asthma will be more likely to develop an allergic airway response to an inhaled food allergen, which might prime for the subsequent development of food allergy to that allergen, when it was ingested.

To test this, we then used these mice to determine whether repeated oral inoculation with egg white would induce shock (hypothermia) and diarrhea. No mice developed shock or diarrhea in response to the first 10 oral challenges, with responses first seen to challenge 11. However, as shown in Figure 2, below, mice that had been inoculated intratracheally with either



F*igure 2. Food allergy development in intratracheally primed mice.* BALB/c mice from the experiment shown in Fig. 1 were inoculated by oral gavage (o.g.) 12 times with egg white and tested for development of hypothermia and diarrhea. Cumulative mortality from the 11th and 12th o.g. inoculations was recorded.

egg white or egg white plus house dust mite developed hypothermia (shock) and often diarrhea in response to ingestion of egg white; anaphylaxis was lethal for approximately 25% of these mice. Surprisingly, disease development was equally severe in the mice that had initially been inoculated intratracheally with egg white or egg white plus house dust. Although this study needs to be repeated, it suggests that the severity of the allergen-specific lung disease is not a factor in the development of food allergy to the same allergen. More importantly, it demonstrates that we are now able to induce the egg white-specific food allergy that is required for our studies by increasing the number of intratracheal and oral immunizations.

While we were trying to get our main model working again, we also explored a model in which immunologically naïve mice were injected with IgE anti-trinitrophenyl (TNP) monoclonal antibody and challenged by oral gavage (o.g.) with TNP-bovine serum albumin (TNP-BSA). This was repeated twice a week. This was done with the expectation that repeated IgE anti-TNP antibody priming and TNP-BSA challenge would eventually lead to the development of an endogenous IgE anti-TNP-BSA, so that o.g. challenge with TNP-BSA would induce shock and possibly diarrhea even when mice were not pre-treated with IgE anti-TNP. Surprisingly, we found that repeated cycles of IgE anti-TNP priming and o.g. TNP-BSA challenge led to loss of the hypothermia response (Figure 3). The main importance of this is that it demonstrates that

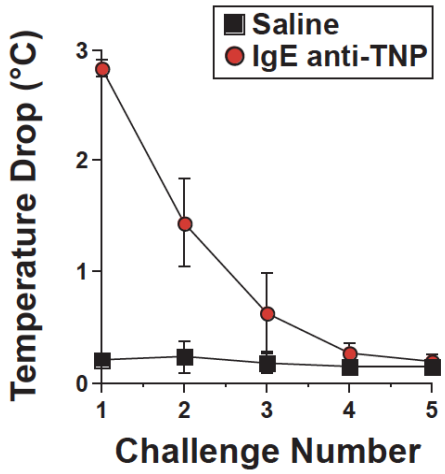


Figure 3. Repeated anti-IgE monoclonal antibody priming and oral antigen challenge leads to desensitization. BALB/c mice were primed i.v. with IgE anti-TNP monoclonal antibody and challenged the next day by oral gavage with TNP-BSA. This was repeated every 4 days for a total of 5 cycles. Rectal temperature was followed for 1 hour after each challenge to detect the development of hypothermia.

intratracheal priming with antigen does not prime for food allergy solely by stimulating an IgE response to ovalbumin. However, it also suggests a novel mechanism for tolerance induction, which we are investigating further. Our leading hypothesis is that tolerance in this model results from induction of a strong IgG1 antibody response to the antigen, which blocks antigen accessibility to mast cell-bound IgE. In this regard, mice that were repeatedly primed with IgE anti-TNP monoclonal antibody and challenged with TNP-BSA developed a serum titer of IgG1 anti-TNP-BSA antibody of 942±99 (mean±SEM, 6 mice/group), vs. <10 in untreated mice.

Finally, in accord with task 3 of Aim 6, we have generated large quantities of monoclonal antibodies to IL-10R, TGF-β and CD25, which will be used, as planned, in our other aims.

Training: My group has been joined by Mr. Vishnu Gudimetla and Dr. Durga Krishnamurthy, PhD. Mr. Gudimetla is a Masters student in the Immunology Graduate Program at the University of Cincinnati. He pays his own tuition and does not receive any salary or benefits from my grant, but contributes by studying the mechanisms responsible for development of food allergy in mouse models. He is responsible for Figure 3 in the “Accomplishment” section of this proposal. Durga Krishnamurthy is doing a post-doctoral fellowship in my lab, working on food allergy. She is responsible for Figures 1 and 2 in the “Accomplishment” section of this proposal; 83% of her salary and benefits are paid by this grant. Both Mr. Gudimetla and Dr. Krishnamurthy meet with me at least once a week to review data, make plans for experiments and go over presentations. Both participate in our weekly lab meeting, journal club and research in progress meeting, as well as the frequent talks by visiting professors. In addtion, Mr. Gudimetla takes didactic courses in Immunology and Molecular Biology at the University of Cincinnati.

Result Dissemination: We are currently discussing our results at local meetings and expect to present them at national scientific meetings and publish them in scientific journals when we have accumulated sufficient data to do that. We have no objections to dissemination of the results of this report by the DOD.

Plans for the Next Reporting Period: Now that our basic assay is working again, we plan to perform those procedures that were originally scheduled for year 1 (Aim 1, tasks 1 and 2 and Aim 2) and begin work on Aim 1, task 3 and Aims 3 and 4.

4. Impact:

On Principal Discipline: We expect that the results of our ongoing and planned studies will increase the appreciation that allergy that involves one organ (in our case, the airways) can increase the likelihood of developing allergy that involves additional organs. More specifically, our work should deepen understanding of how inhaled allergens can promote the development of food allergy to cross-reactive allergens. If our results support our hypotheses, they will suggest that early oral intake of potential food allergens will decrease the likelihood of development of food allergy and respiratory allergy to those allergens and to cross-reactive allergens.

On Other Disciplines: Nothing to report.

On Technology Transfer: Nothing to report.

On Society Beyond Science and Technology: Nothing to report.

5. Changes/Problems:

Changes in Approach and Reasons for Change: As noted below, our basic experiment, that is the root of nearly all of our studies, stopped working during year one, possibly because construction adjacent to our animal colony increased the stress of mice. Mice still developed allergic lung disease after being inoculated intratracheally with egg white, but failed to develop shock or diarrhea when subsequently challenged with egg white by oral gavage. Now that the noisy phase of construction is complete, our mice are once again developing shock and diarrhea in response to oral gavage with egg white after being initially inoculated intratracheally with egg white; however, it now requires 12 oral gavages over 4 weeks instead of 6 oral gavages over 2 weeks. This increases our costs (personnel and mouse per diem) per experiment and delays the completion of each experiment. We are continuing to experiment with minor changes to our protocol, including the addition of egg yolk to egg white, to see if this will facilitate the more timely development of clinical features of food allergy. We do not think these changes will require any changes in our IACUC or other animal protocols, but can amend them if necessary.

Actual or Anticipated Problems or Delays and Actions or Plans to Resolve them: Please see above section.

Changes that had a significant impact on expenditures: The problems noted above have neither increased nor decreased expenditures, but may decrease the amount of work accomplished for the planned cost because of the longer duration of experiments and the use of mice and personnel in experiments that did not work.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: None.

6. Products:

Publications, conference papers, and presentations: None to report.

Websites or other Internet sites: None to report.

Technologies or Techniques: None to report.

Inventions, patent applications, and/or licenses: None to report.

Other products: None to report:

7. Participants and other collaborating organizations:

Individuals who have worked on the project:

Name: Fred Finkelman, M.D.
Project Role: PI
Nearest person months worked: 3.0
Contribution to Project: Directs project; plans experiments, interprets results, writes papers and reports.
Funding Support: This grant

Marat Khodoun, Ph.D.
Project Role: co-Investigator
Nearest person months worked: 3.72
Contribution to Project: Prepares reagents, inoculates and tests mice for food allergy; contributes to planning and interpreting experiments.
Funding Support: This grant

Durga Krishnamurthy, Ph.D.
Project Role: co-Investigator
Nearest person months worked: 10.0
Contribution to Project: Inoculates and tests mice for food allergy; evaluates mice for tolerance induction; contributes to planning and interpreting experiments.
Funding Support: This grant.

Vishnu Gudimetla, B.A.
Project Role: Masters' Degree student
Nearest person months worked: No support from grant
Contribution to Project: Studies the mechanisms by which intratracheal inoculation with allergen primes for the development of food allergy to that allergen.
Funding Support: His own tuition payments; no salary

Charles Perkins, B.A.
Project Role: Research Assistant
Nearest person months worked: 12.0
Contribution to Project: Inoculates mice intratracheally and performs studies of lung function, assists with studies of intestinal function; prepares monoclonal antibodies.

Funding Support: This grant

Crystal Potter, B.A.
Project Role: Research Assistant
Nearest person months worked: 3.0
Contribution to Project: Breeds and PCR types mice
Funding Support: This grant

Changes in active other support of the PI and senior/key personnel since the last reporting period.

The PI has received two new grants since the start of his DOD grant:

1. Food Allergy Research and Education Proposal (F. Finkelman, P.I.) 6/14–5/17 3.0 calendar
   Food Allergy Research and Education (non-profit foundation) $218,401. Non-VA Effort
   Rapid suppression of food allergy with anti-FceRIa antibody
   This project uses rapid desensitization with anti-FceRIa mAb to suppress established models of food allergy in conventional and humanized mice.

2. 1R01AI113162-01 (Fred Finkelman, P.I.) 07/15/14 – 06/30/18 3.0 calendar
   NIH/NIAID $250,000.00 Non-VA effort
   Suppression of IgE-mediated disease by polyclonal rapid desensitization
   This project will develop a novel approach to rapidly suppress human IgE-mediated allergy.

Marat Khodoun also receives salary support from both of these grants.

Other organizations involved as partners: None to report.

8. Special reporting requirements: None

9. Appendices: None